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XXXIV.—*A Novel Instance of the Production of Fermentation by the Presence of Infusoria capable of existing without free Oxygen and deprived of all Access of Atmospheric Air.* By M. L. Pasteur.

Such is the subject of a short paper addressed by M. Pasteur to the French Academy of Sciences, and published in the 'Comptes Rendus' for March 1863.

About two years previously, he communicated a note to the same learned body respecting the existence of infusory animalcules possessing the two properties of living without free oxygen, and of acting as ferments*. "These were," he says, "the first known examples of an animal ferment, as well as of animals capable of living and of indefinite self-multiplication, without contact with atmospheric air, whether in the gaseous state or held in solution.

"The infusory animalcules in question constitute the ferment in butyric fermentation, which has hitherto been explained to take place by the agency of plastic azotized matters, more or less changed by contact with the air, upon sugar or lactic acid, and by the supervention of an internal molecular action giving rise to the phenomena of fermentation.

"I believe I have proved that such a theory, which is applied indeed in explanation of all kinds of fermentation, properly so-called, is inadmissible, and that an albuminoid matter never constitutes a ferment, but that the true ferment (as in butyric fermentation, for example) is an organized being belonging to the Vibrios, derived from the air and present in the fermentable substance.

"I am now able to add another example—viz. the fermentation of tartrate of lime, determined in precisely the same way

* See Annals, April, 1861, p. 343.

by the presence of an infusory animalcule, existing without free oxygen, belonging also to the genus *Vibrio*, though very different, at least in external aspect, from the animalcule of butyric fermentation.

"To be brief, I will at once adduce a decisive experiment in proof of this statement. I place in an aqueous solution of tartrate of lime a minute portion (some millièmes) of phosphate of ammonia or of alkaline and earthy phosphates, either artificially prepared, or derived from the ashes of the yeast of beer or the ashes of infusory organisms. (I prefer the ashes obtained by the combustion of organisms analogous to those whose development is sought, in order to be more certain that no useful principle, known or unknown, is omitted. It is probably as well to add some traces of sulphate of lime and of ammonia.)

"The vessel used is a glass phial, flat at the bottom, and having a curved glass tube fitted in its narrow neck. The tartrate of lime is introduced, the phial filled up with pure water, and then placed in a chloride-of-calcium bath with the end of the curved tube immersed in a vessel of boiling water. Its contents are made to boil, in order to expel all the air held in solution; and when this is effected, the surface of the water under which the end of the curved tube opens is covered by a thick layer of oil. The whole apparatus is then left to cool for some hours. Under these conditions, the tartrate shows no sign of fermentation; but if a small quantity of the Infusoria obtained from a spontaneously fermenting portion of tartrate of lime be quickly introduced within the phial, and the little water displaced in this process be as quickly replaced by some of the water deprived of its air by boiling, then it is found that the introduced Infusoria rapidly multiply, and the tartrate progressively disappears until entirely removed, all air having been in the meanwhile excluded by the curved tube of the phial being kept under the water, or, better still, under the surface of a mercury bath.

"The tartrate was replaced by a deposit consisting solely of the bodies of Vibrios, of about $\frac{1}{1000}$ millimètre in diameter, but so variable in length that some measured $\frac{1}{20}$ millimètre. Like all Vibrios, they are reproduced by fission; and during the act of fermentation, the minutest quantity of the deposit formed by them showed them in more or less rapid and writhing movement.

"The fermentation of tartrate of lime, therefore, whatever its intimate cause may be, is set up by the presence of Infusoria having the property of living without free oxygen and without contact with atmospheric air.

"It may undoubtedly be objected that, at the moment when

the ferment has been added, I have failed to prevent the contact of the air with the solution experimented upon. But I will now demonstrate that the very strict precautions I have taken to obviate the contact of oxygen or of air are really uncalled for. The following observations will also afford a reply to the question why the germs of Infusoria which not only live without air, but are actually destroyed by it (as happens also with the butyric Infusoria), may spontaneously originate in liquids which under the circumstances of ordinary fermentation are exposed to the atmosphere.

"Resuming the phial filled with water, and containing the tartrate of lime and the phosphates, and having the bent tube luted in its neck also filled with water (which I will suppose to be ordinary distilled water undeprived of its air by boiling), and with its free extremity plunged under mercury, it will be found by experiment that, without adding any ferment, fermentation of the tartrate of lime takes place at the end of a few days, and that a multitude of animalcules are found living in the phial, though deprived of oxygen.

"How this happens it is easy to conceive; for in all such cases the smallest Infusoria, such as *Monas, Bacterium termo*, &c., develope themselves in the aërated distilled water, which contains in solution traces of ammonia, of phosphate and carbonate of lime, together with oxygen gas; this last they appropriate to themselves with incredible rapidity, until it is ultimately used up, replacing it by carbonic acid in somewhat larger volume. This result is accomplished in from twenty-four to thirty-six hours at most, at a temperature of 25° to 30° Cent.; and it is not till then that the Infusoria of fermentation make their appearance, which have no need of oxygen for their existence. The question, therefore, why animalcules which do not require oxygen to carry on life, and to which air is destructive, should arise under the conditions assumed, is thus at once and naturally answered. They originate in sequence to a former generation of organisms which quickly abstract the relatively considerable quantity of oxygen in the fluid, and leave it completely destitute of that element.

"I shall shortly revert to this very general fact of the succession of organisms which consume oxygen, and of such as do not, at least in a free state.

"In the instance under consideration, it is easy to comprehend the facility with which spontaneous fermentation of tartrate of lime may be set up, whenever special precautions are not taken to prevent the access of the germs disseminated through the air, or those in the dust deposited from the air on all objects. It is equally easy to understand the fermentation of tartrate of lime

in liquids freely exposed to the air, provided the layer of fluid is of sufficient thickness. It may under such circumstances be shown that those Infusoria which consume oxygen gas multiply at the surface; whilst those are developed in the liquid strata beneath which do not require this gas for their existence, and these are at the same time preserved from its injurious contact by the former class of beings.

"In fine, there is no need to resort to any artificial measures to deprive fluids of their oxygen gas. All the precautions I adopted in my experiments for this purpose were wholly superfluous. The abstraction of the oxygen is naturally effected, as a matter of course, before fermentation begins, in every instance of spontaneous fermentation.

"The nature of the experiments above detailed, and the composition of the materials employed in them, deserve particular notice when we come to inquire what may be the primary cause of the fermentation. I have referred to the prevalent theories as requiring, as indispensable for the act of fermentation, the concurrence of albuminoid matter and of a ferment. For my part, I recognize their presence to be not absolutely necessary, but useful, inasmuch as they supply a certain material for the action of the ferment, which is itself an organized being whose germ cannot develope or reproduce itself except in the presence of nitrogen and phosphates. These are especially the kind of suitable materials that the ferment obtains from the presence of albuminoid substances. This theory is so true in its application, that, as before seen, the azotized plastic matter may be entirely dispensed with, and its place supplied by an ammoniacal salt mingled with alkaline and earthy phosphates.

"But it further results from the composition of the fluid just spoken of holding a tartrate in solution, that the sole carbonaceous material for fermentation is the tartaric acid, or the fermenting body itself. Hence this further result follows, that at the least the animalcule derives in the first place all its carbon from the fermentable matter. There is no question, if preconceived notions relative to the cause of fermentation be laid aside, that under the conditions which we have described, the ferment obtains its nutrition at the expense of the fermenting material, and that so long as the life of the infusory organism lasts, so long does a transfer of matter go on from the fermenting substance to that which provokes its transformation. The hypothesis of a purely catalytic action, or of simple contact, consequently cannot be admitted as true, any more than the opinion, to be afterwards combated, that the nature of a ferment is exclusively found in dead albuminoid matter.

"It must be granted that the fact of the nutrition of the fer-

ment advancing at the expense of the fermentable material does not show why the Vibrios must be the ferment. We know, indeed, that the habitual mode of action of animals and plants on the substances from which they directly derive their nourishment is not associated with a process of fermentation, properly so called, of those substances. Yet it must be borne in mind, in making any comparison between organisms previously known and those which I have for the first time described, that animalcular ferments present this peculiar physiological property, heretofore overlooked, that they live and multiply without the presence of free oxygen.

"We are therefore led to associate the fact of nutrition attended by fermentation with that of nutrition without the consumption of free oxygen gas. Herein, no doubt, lies the secret of the mysterious character of all fermentation, rightly so called, and possibly also that of many normal and abnormal actions in the organization of living beings. If any doubts yet remain on these points, I trust to remove them by future researches which I hope to lay before the Academy.

"Henceforward it may be asserted that there are two modes of life among inferior organisms—the one requiring the presence of free oxygen, the other carried on without contact with this gas, and always attended by the phenomena of fermentation.

"As to the number of organisms capable of living deprived of air, and of setting up fermentation, I regard it as considerable, whether we look to those having no inherent power of self-movement, in other words, vegetable beings, or to those which have apparent voluntary motions, or animals.

"I hope, in fact, to demonstrate in a subsequent communication, that infusory animalcules living without the access of free oxygen are the ferments of putrefaction when this act proceeds without contact with air; and that there are other animalcular ferments of putrefaction under exposure to air, which are found associated with Infusoria or Mucors, that consume the free oxygen and fulfil the double purpose of agents of combustion with reference to the organic material, and of agents of preservation for the infusorial ferments, by protecting them from the contact of the oxygen of the air."

The results now described apply exclusively to the case of the simple tartrate of lime; but the author has a series of researches which extend them to the other combinations of lime and tartaric acid, and which he promises shortly to send to the Academy of Sciences.

J. T. Arlidge.